Attorney Docket No. 5051-338CT

Applicant Serial No: 09/963,340 Filed: September 24, 2001

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IN THE SPECIFICATION

Please amend the specification as follows.

On page 5, lines 8-15, please amend the paragraphs as follows.

Figure 5 compares nicotine levels and the relative steady-state *NtQTP1* mRNA levels in *Nic1* and *Nic2* tobacco mutants: wild-type Burley 21 (*Nic1/Nic1 Nic2/Nic2*); *Nic1* Burley 21 (*nic1/nic] Nic2/Nic2*); *Nic2* Burley 21 (*Nic1/Nic1 nic2/nic2*); and *Nic1* Nic2 Burley 21 (*nic1/nic1 nic2/nic2*). SolidForward slash bars indicate mRNA transcript levels; hatchedback slash bars indicate nicotine levels.

**Figure 6** charts the relative levels of *NtQPT1* mRNA over time in topped tobacco plants compared to non-topped control plants. <u>SolidForward slash</u> bars indicate mRNA transcript levels; hatchedback slash bars indicate nicotine levels.

On page 23, lines 9-13, please amend the paragraph as follows.

TobRD2 steady-state mRNA levels were examined in Nic1 and Nic2 mutant tobacco plants. Nic1 and Nic2 are known to regulate quinolate phosphoribosyl transferase activity and putrescence methyl-transferase activity, and are co-dominant regulators of nicotine production. The present results are illustrated in Figures 5A and 5B showFigure 5, which shows that TobRD2 expression is regulated by Nic1 and Nic2.

On page 23, line 17 through page 24, line 6, please amend the paragraphs as follows.

Four Burley 21 tobacco lines (nic) were grown from seed in soil for a month and transferred to hydroponic chambers in aerated nutrient solution in a greenhouse for one month. These lines were isogenic, except for the two low-nicotine loci, and had genotypes of *Nic1/Nic1 Nic2/Nic2*, *Nic1/Nic1 nic2/nic2*, *nic1/nic1 Nic2/Nic2*, *nic1/nic1 nic2/nic2*. Roots were harvested from about 20 plants for each genotype and pooled for RNA isolation. Total RNA (1 fug) from each genotype was electrophoresed through a 1% agarose gel containing 1.1 M formaldehyde and transferred to a nylon membrane according to Sambrook et al. (1989). The membranes were hybridized with <sup>32</sup>P-labeled TobRD2 cDNA fragments. Relative intensity of TobRD2 transcripts were measured by densitometry.

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**Figure 5** (solid bars)(forward slash bars) illustrates the relative transcript levels (compared to *Nic1/Nic1 Nic2/Nic2*) for each of the four genotypes. The relative nicotine content (compared to *Nic1/Nic1 Nic2/Nic2*) of the four genotypes is shown by the hatched back slash bars.

Figure 5 graphically compares the relative steady state TobRD2 5 mRNA level, using the level found in wild-type Burley 21 (Nic1/Nic1 Nic2/Nic2) as the reference amount. TobRD2 mRNA levels in Nic1/Nic2 double mutants were approximately 25% that of wild-type tobacco. Figure 5B5 further compares the relative levels of nicotine in the near isogenic lines of tobacco studied in this example (solidforward slash bars indicate TobRD2 transcript levels; hatched back slash bars indicate nicotine level). There was a close correlation between nicotine levels and TobRD2 transcript levels.

On page 24, line 15 through page 25, line 2, please amend the paragraphs as follows.

Tobacco plants (*N tabacum* SRI) were grown from seed in soil for a month and transferred to pots containing sand. Plants were grown in a greenhouse for another two months until they started setting flowers. Flower heads and two nodes were then removed from four plants (topping). A portion of the roots was harvested from each plant after the indicated time and pooled for RNA extraction. Control plants were not decapitated. Total RNA (1µg) from each time point was electrophoresed through a 1% agarose gel containing 1.1M formaldehyde and transferred to a nylon membrane according to Sambrook, et al. (1989). The membranes were hybridized with <sup>32</sup>P-labeled TobRD2 cDNA fragments. Relative intensity of TobRD2 transcripts were measured by densitometry. **Figure 6** illustrates the relative transcript levels (compared to zero time) for each time-point with topping (solidforward slash bars) or without topping (hatchedback slash bars).

Relative *TobRD2* levels were determined in root tissue over 24 hours; results are shown in **Figure 6** (solid<u>forward slash</u> bars indicate *TobRD2* transcript levels in topped plants; <u>hatchedback</u> <u>slash</u> bars indicate the *TobRD2* transcript levels in non-topped controls). Within six hours of topping of tobacco plants, mRNA levels of *TobRD2* increased approximately eight-fold in the topped plants; no increase was seen in control plants over the same time period.